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INHIBITION OF GASTRIC SECRETION BY BACTERIAL LIPOPOLYSACCHARIDE IN THE RAT

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Bacterial lipopolysaccharide (LPS) provoked an inhibition of gastric secretion in the rat. Reserpine and the catecholamine-synthesis inhibitors α-methyldopa and diethyl dithiocarbamate blocked this action of LPS, although adrenergic blocking agents or adrenalectomy were without effect. Direct stimulation of gastric secretion by carbachol also opposed the LPS effect, whereas central parasympathetic stimulation by 2-deoxy-D-glucose did not.

Lipopolysaccharide Gastric secretion Autonomic nervous system

1. INTRODUCTION

Endotoxins and purified lipopolysaccharides (LPS) derived from Gram-negative bacteria are potent inhibitors of gastric secretion. This has been shown in the dog (Blickenstaff and Grossman, 1950; Wyllie et al., 1967) and in the rat (Olson et al., 1954; Brodie and Kundrats, 1964; Baume et al., 1967).

Alterations of gastric secretion are usually the consequence of changes in parasympathetic or sympathetic activities (Jacobson, 1965; Bass and Patterson, 1967). LPS produces marked adrenergic stimulation (Gilbert, 1960) and may act, therefore, on gastric secretion via a stimulation of the sympathetic nervous system or release of catecholamines from the adrenal medulla into the circulation. However, LPS could also inhibit the parasympathetic nervous system or directly influence the secretory cells of the gastric mucosa. Hyperthermia can be excluded as the cause of inhibition of gastric secretion by LPS in the rat because in this species LPS does not produce hyperthermia (van Miert and Frens, 1968; Filkins and Di Luzio, 1968).

Therefore, the influence of blockade of the sympathetic nervous system and stimulation of the parasympathetic system at different levels were studied on the inhibition of the gastric secretion by LPS.

2. METHODS

Male Wistar rats weighing 125–225 g were used. Gastric juice was collected according to the method of Shay et al. (1954)*. The rats were kept in a metabolic cage and fasted for 24–30 hr prior to the pyloric ligature, whereas they received water *ad libitum*. Immediately following the operation LPS or sterile 0.9% saline was given in a volume of 0.1 ml/100 g body weight via a lateral tail vein. Three hours later the animals were killed, the stomachs removed and washed out with 4 ml distilled water. After centrifuging (10 min, 1000 *g*), the volumes of

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* This is a simple method, suitable for indicating a possible mode of action of endotoxin.

supernatant and solid matter were noted. If the volume of the supernatant was found to be less than 4 ml or the volume of the solids was found to be more than 1.0 ml, the animal was excluded from the experiment (Shay et al., 1954). The pH of the supernatant was measured and the titratable acidity was determined by titration with 0.1 N NaOH using phenolphthalein as indicator.

Adrenalectomy was performed one week before pyloric ligation, using the bilateral dorsolateral approach. The rats received thereafter 0.9% saline as drinking water. To maintain gastric secretion of the adrenalectomized rats (Cooke et al., 1966) the animals were treated with cortisol (twice daily, s.c. 100 μg/rat).

The following substances were used: LPS *Escherichia coli* $O_{111}B_4$ charge 487989 (Difco Lab. Detroit, Mich. USA) 1 μg/kg i.v., 2-deoxy-D-glucose 100 mg/kg s.c., carbachol 0.2 mg/kg s.c. and propranolol (Inderal®) 1 mg/kg s.c. immediately after pyloric ligation; dibenamine 5 mg/kg s.c. one hour before the LPS injection; reserpine (Serpasil®) 4 mg/kg s.c. daily during 3 days and 0.4 mg per animal at the time of the ligation; sodium diethyl dithiocarbamate 500 mg/kg s.c. 10 hr before the ligature and at the time of the LPS injection; α-methyldopa (Aldomet®) 200 mg/kg s.c. 4 hr before the ligature and at the time of the LPS injection.

In the different experiments the animals were treated in random order. Results are expressed as mean ± standard error of the mean (S.E.). Statistical analysis of the data was performed using Wilcoxon's two sample test (Wilcoxon, 1945).

3. RESULTS

As shown in table 1, LPS (1 μg/kg, i.v.) provoked an inhibition of the volume, pH, total acid output and acid concentration of the gastric juice in the rat. Similar results were found in all separate experiments of this table.

Pretreatment with the adrenergic blocking agents dibenamine or propranolol (table 1) and adrenalectomy (table 2) did not prevent the inhibition of gastric secretion by LPS. Dibenamine itself diminished the volume and total acid output of the gastric juice ($p<0.05$).

Reserpine pretreatment (table 1) stimulated the gastric juice volume ($p<0.05$). LPS still reduced the secretion of the volume and total acid of the gastric juice ($p<0.02$), but the reduction was significantly less than in the saline-LPS group ($p<0.02$).

The inhibitors of catecholamine-synthesis, α-methyldopa (table 1) and diethyl dithiocarbamate (table 2), depressed the gastric secretion strongly

Table 1
Influence of dibenamine, propranolol, reserpine, α-methyldopa and carbachol on the inhibition of the gastric secretion by LPS (1 μg/kg) in the rat.

		Gastric volume (ml/kg b.w.)	pH	Total acid output (mEq/100 g b.w./3 hr)	Acid concentration (mEq/l)
Saline	+ saline	27 ± 3 * (9)	2.4 ± 0.7	218 ± 31	80 ± 7
	+ LPS	12 ± 1 (9)	4.8 ± 0.6	59 ± 8	53 ± 13
Dibenamine	+ saline	17 ± 2 (5)	3.0 ± 0.6	119 ± 20	69 ± 7
	+ LPS	8 ± 1 (6)	5.0 ± 0.6	35 ± 5	47 ± 8
Propranolol	+ saline	26 ± 5 (5)	2.2 ± 0.3	206 ± 48	79 ± 9
	+ LPS	17 ± 6 (4)	4.1 ± 1.1	106 ± 46	63 ± 8
Reserpine	+ saline	37 ± 3 (4)	1.8 ± 0.1	265 ± 46	70 ± 6
	+ LPS	23 ± 3 (5)	2.5 ± 0.4	124 ± 25	53 ± 4
α-Methyldopa	+ saline	8 ± 1 (5)	3.4 ± 0.5	77 ± 18	99 ± 13
	+ LPS	9 ± 1 (5)	3.3 ± 0.3	72 ± 10	80 ± 8
Carbachol	+ saline	40 ± 3 (5)	1.8 ± 0.1	328 ± 55	82 ± 9
	+ LPS	41 ± 3 (5)	1.9 ± 0.1	242 ± 36	58 ± 7

* Mean ± standard error of the mean.
Number of animals used in each group is given in brackets.

Table 2
Influence of diethyl dithiocarbamate (DDC), adrenalectomy and 2-deoxy-D-glucose (2-DG) on the inhibition of the gastric secretion by LPS (1 μg/kg) in the rat.

		Gastric volume (ml/kg b.w.)	pH	Total acid output (mEq/100 g b.w./3 hr)	Acid concentration (mEq/l)
Saline	+ saline	16 ± 1 * (5)	2.0 ± 0.1	173 ± 32	104 ± 14
	+ LPS	4 ± 0 (4)	5.3 ± 0.2	27 ± 3	74 ± 12
DDC	+ saline	6 ± 2 (5)	2.3 ± 0.3	65 ± 20	99 ± 9
	+ LPS	9 ± 3 (5)	2.5 ± 0.4	81 ± 24	98 ± 3
Sham	+ saline	42 ± 6 (3)	2.1 ± 0.2	407 ± 105	95 ± 12
	+ LPS	24 ± 3 (4)	3.7 ± 1.1	195 ± 56	78 ± 18
Adrenalectomy	+ saline	31 ± 2 (5)	1.9 ± 0.1	277 ± 58	87 ± 14
	+ LPS	17 ± 1 (6)	4.0 ± 0.4	91 ± 15	53 ± 6
Saline	+ saline	21 ± 2 (10)	2.2 ± 0.1	194 ± 26	94 ± 5
	+ LPS	8 ± 2 (7)	4.2 ± 0.7	100 ± 29	69 ± 18
2-DG	+ saline	30 ± 6 (11)	2.0 ± 0.1	280 ± 49	94 ± 6
	+ LPS	12 ± 2 (7)	4.2 ± 0.7	55 ± 15	44 ± 5

* Mean ± standard error of the mean.
Number of animals used in each group is given in brackets.

($p<0.01$) and no further inhibition was produced by LPS. In the diethyl dithiocarbamate-LPS group, total acid output was found to be significantly higher ($p<0.02$) (and the pH significantly lower ($p<0.02$)) than in the saline-LPS group. The α-methyldopa-LPS group had a significantly higher ($p<0.05$) acid concentration and lower ($p<0.05$) pH than the saline-LPS group. Thus, the catecholamine-synthesis inhibitors did not reduce gastric secretion to a minimum level.

Carbachol (table 1) stimulated the volume ($p<0.01$) and total acid ($p<0.05$) output of the gastric juice. In the animals pretreated with carbachol, LPS only inhibited the acid concentration of the gastric juice significantly ($p<0.05$) but there was a concomitant slight decrease of the total acid output.

2-Deoxy-D-glucose (table 2) stimulated the gastric juice volume ($p<0.05$) and increased the total acid output, but did not affect the inhibition of gastric secretion by LPS.

4. DISCUSSION

The present experiments confirm the studies of Olson et al. (1954), Brodie and Kundrats (1964) and Baume et al. (1967), that LPS is an inhibitor of gastric secretion in the rat. Adrenalectomy and the adrenergic blocking agents dibenamine and propranolol did not affect the action of LPS, whereas the catecholamine-depletor, reserpine, partially and the catecholamine-synthesis inhibitors, α-methyldopa and diethyl dithiocarbamate, completely opposed the inhibitory action of LPS. Direct stimulation of gastric secretion by carbachol also completely prevented the effect of LPS, whereas central parasympathetic activation of gastric secretion by 2-deoxy-D-glucose did not.

Because carbachol prevented most of the actions of LPS, an inhibitory effect of LPS on the cholinergic receptors in the gastric mucosa can probably be excluded. 2-Deoxy-D-glucose pretreatment, however, did not affect the action of LPS. Therefore, an effect of LPS more centrally in the parasympathetic pathway remains possible. Perhaps the inhibitory effect of LPS was much greater than the stimulant action of 2-deoxy-D-glucose on the parasympathetic system. This might also explain this finding.

The action of LPS was unlikely to be exerted mainly through a catecholamine-releasing effect on the adrenal medulla, because adrenalectomy did not influence the action of LPS. It is, however, possible that the LPS effect is mediated by an increase in adrenergic neuronal activity, since some inhibitors of

this activity (α-methyldopa, diethyl dithiocarbamate and reserpine) prevented the effect of LPS on gastric secretion.

That the adrenergic receptor blocking agents, dibenamine and propranolol, did not prevent the inhibition of the gastric secretion by LPS weakens this conclusion although responses to circulating catecholamines are inhibited more effectively by adrenergic blocking agents than are the responses to the neurotransmitter released locally at the sympathetic nerve endings (Goodman and Gilman, 1965). Therefore it is still possible that LPS acts on local catecholamine release.

The low level of gastric secretion caused by α-methyldopa and diethyl dithiocarbamate might also explain the inhibition of the LPS effect. However, since secretion stayed significantly higher than in the control-LPS groups, inhibition of catecholamine-synthesis inhibition is also a factor in the reduction in the effect of LPS. That dibenamine, α-methyldopa and diethyl dithiocarbamate depress gastric secretion is difficult to reconcile, with the hypothesis that the sympathetic nervous system depresses gastric secretion.

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